University of Applied Sciences and Arts Northwestern Switzerland
School of Life Sciences

PAL SYSTEM
Ingenious sample handling

Automated Sample Preparation of Whole Blood for Therapeutic Drug Monitoring and Diagnostics by LC-MS using a Commercial Autosampler

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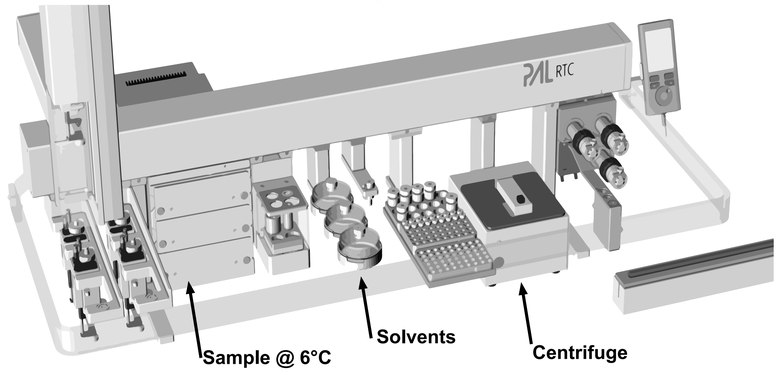
Introduction

Automated sample preparation reduces the costs per sample and avoids sample handling errors. This is especially important in therapeutic drug monitoring or diagnostics based on blood samples. The use of robots is well established in these fields since a long time. Usually expensive and highly specialized pipetting robots are used for a high number of samples. However, most of these systems are not designed with a direct interface for LC-MS applications.

In this project the parameters necessary to automatically prepare whole blood samples for online LC-MS applications in the field of diagnostics and TDM have been investigated. A strategy and the most important parameters are shown for the optimization of a PAL RTC autosampler for the preparation of whole blood.

Materials and Methods

An integrated system consisting of a PAL RTC autosampler equipped with a 1 mL syringe, a LC-MS injection tool, a vortexer and a centrifuge was used (see below).

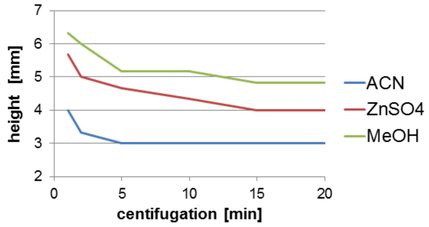


Tested laboratory blood was supplied by blood donation and applied into 1.5 ml standard LC-vials with magnetic caps for vial transport. Methanol, acetonitrile, 0.1 mol/L ZnSO4 solutions in methanol and a mix of isopropanol/water have been tested to achieve protein precipitation. Blood volumes in the range of 50 to 200 µL were investigated at centrifugation times of 1 to 20 minutes (3000 g).

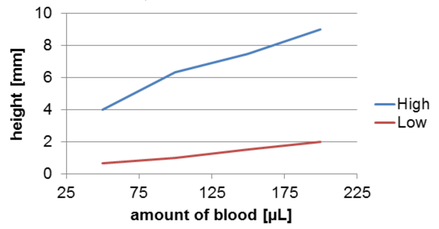
Results - Precipitation and Exctraction

Precipitating and extracting depends on the solvent used and is alwas dependet on the metabolites analysed. The target is the best compromise between efficiency of extraction, pellet size and clear supernatatant.

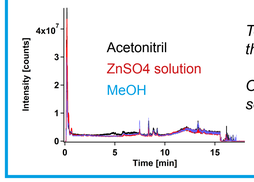
Precipitated and centrifuged



Pellet height depending on the duration of centrifugation and solvent used for 50 µL blood



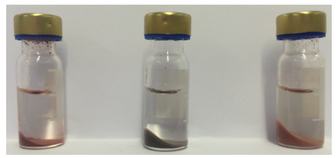
Pelet hight for 0.1 mol/L ZnSO4 solution in MeOH at 10 min centrifgation. Blue is the high point and red the low one of the pelet.



Total ion chromatogram (TIC) of the solvent after precipitation.

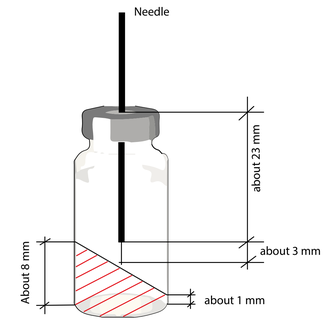
Only minor differences are observesd.

Pelet of different solvents for 50 µL blood



Acetonitril | 0.1 mol/L ZnSO4 in methanol | Methanol

Needle penetration parameters

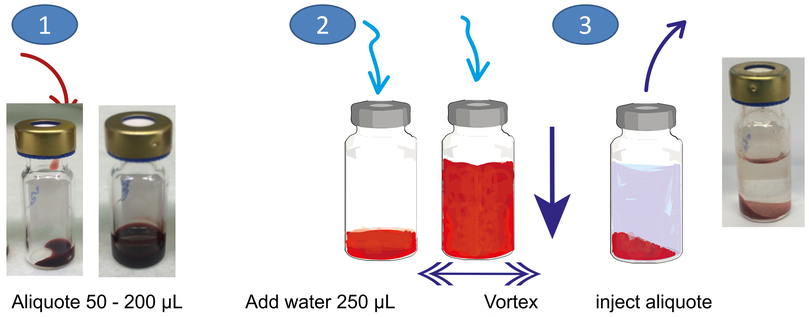


To avoid clocing of the needle should not penetrate below 8 mm up to 100 µL. A total sample volume of 200 µL blood (per 1.5 mL vial) causes a pellet which is of acceptable height (10 mm maximum).

Sample Preparation Procedure

Since the PAL operates with standard vials its necessarry to modify the sample preparation steps accordingly

50-200µL blood → + 250µL H_2O → + 750µL solvent → 1. Vortex 2. Centrifuge → Inject 2µL supernatant



Aliquote 50 - 200 µL blood into a HPLC vial | Add water 250 µL 750 µL crashing solvent | Vortex Centrifuge | inject aliquote

Results - Diagnostics

Metabolites, which are of diagnostic value, are shown as a typical example. The method is comparable to those used in newborn screening based on dried blood spots [3]

Euent A: Water 0.1% Formic acid , 2 mM ammonium fluoride

Eluent B: Methanol 0.1% Formic acid, 2 mM ammonium fluoride Isocratic 35% A

Flow: 0.6 ml/min *Runtime*: 5 min

Column: Waters Xbridge C18 3x50 mm 3.5 µm

Blood Volume: 50 µL

Injection volume: 2 µL

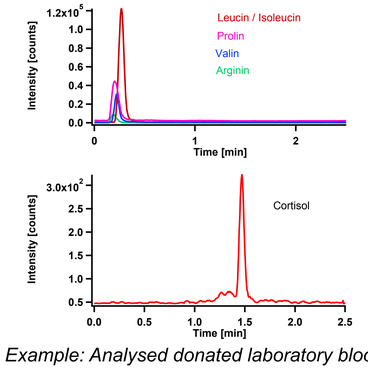
Crashing solvent: Acetonitril

Vortex time: 90 s

Centrifugation time: 3 min

MS Parameter: Mode: Positive

	Fragmentor	Parrent ion	Colision	Product ion
Cortisol	120 V	263.2	25	121.1
C4-carnitine	120 V	232.1	20	85.1
C3-carnitine	100 V	218.1	20	85.1
C2-carnitine	100 V	204.1	20	85.1
Arginin	100 V	175.1	20	70
Carnitin	100 V	162.1	25	85.1
Leucin	80 V	132.1	10	86
Valin	80 V	118.1	10	72
Prolin	100 V	116.1	0	116.1



Example: Analysed donated laboratory blood

Results - Therapeutic Drug Monitoring (TDM)

Diclophenac

Diclophenac is a very typical pain killer. Although it is not very important for general TDM it is a very typical molecule for small molecule with active properties.

Method: Eluent A: Water 0.1% Formic acid / Eluent B: Acetonitril 0.1% Formic acid // *Flow*: 0.5 ml/min // *Runtime*: 5 min

Column: Waters Xbridge C18 3x50 mm 3.5 µm
MS Parameter: Mode: negative Gradient:

Fragmentor	Parrent ion	Colision	Product ion
50 V	294	50	214

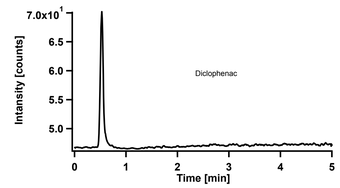
Time	%A
0	70
0.2	70
2	0
3.9	0
4	70

Blood Volume: 50 µL

Injection volume: 2 µL

Precipitation solvent: Acetonitril

Vortex time: 90s Centrifugation: 10 min



Example 300 ng/ml Diclophenac in whole blood (spiked)

Carbamazepine

Carbamazepine is a representative of an anti-epileptic drug and very important to monitor in blood to set a proper and individual dosage. [1]

Method: Eluent A: Water 0.1% Formic acid / Eluent B: Acetonitril 0.1% Formic acid //

Flow: 0.7 ml/min // *Runtime*: 5 min

Column: Waters Xbridge C18 3x50 mm 3.5 µm
MS Parameter: Mode: positive Gradient:

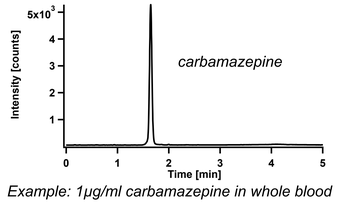
Fragmentor	Parrent ion	Colision	Product ion
135 V	237	18	194

Time	%A
0	80
3	0
4	0
5	0
4.1	80

Blood Volume: 50 µL / Injection volume: 2 µL

Precipitation solvent: Acetonitril

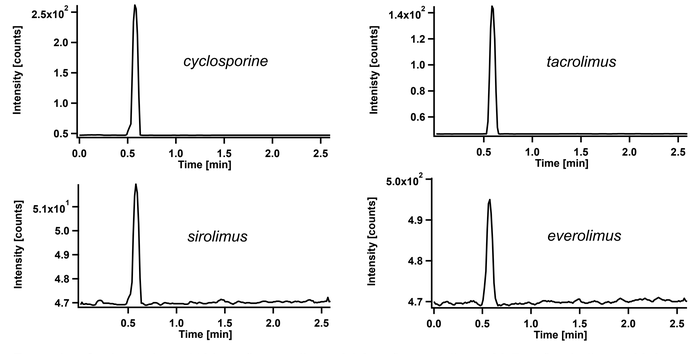
Vortex time: 90s Centrifugation: 10 min



Example: 1µg/ml carbamazepine in whole blood

Immunsuppresiva

Immunosuppressive are measured by the use of an online SPE method, which uses a short column for pre-cleaning and a second column for separation. The method is based on Seger et al. [2]



Example: Cyclosporine: 1100 µg/L, tacrolimus 34.8 µg/L, everolimus 32.7 µg/L, sirolimus, 39.2 µg/L ot of whole blood reference standard by chromsystems (No 0081)

References
[1] Linder et al. Bioanalysis (2015) 4 (16), 2013-2039
[2] Seger et al. Nature Protocols (2009) 4 (4), 526-534
[3] Fingerhut et al. Rapid Communications in Mass Spectrometry (2014) 28 (8), 965-973

Poster Reprint